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(54) Title: ASSAY FOR HIV-1 GROUP O USING AT LEAST ONE GP41 PEPTIDE

(57) Abstract

An antigen that comprises or consists of a peptide having the following sequence: DRQISNISSTIYEEIQKAQVQQEQNEKKLLELD (SEQ. ID. NO. 1) or that has the antigenic properties of that peptide, may be used to detect antibodies to HIV-1 group O. It is advantageous to incorporate the antigen in an HIV-1 assay, preferably also with an antigen from the presumptive immunodominant region of the transmembrane glycoprotein gp41 of HIV-1 group O. HIV-2 antigens may also be incorporated in the assay.

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Assay for HIV-1 group O using at least one GP41 peptide.

The present invention relates to a method for detecting antibodies to HIV-1 group O, and to antigens for use in such a method.

Most commercially available assays for HIV detect antibodies to the virus rather than viral antigens themselves. Current commercial anti-HIV immunoassays detect antibodies against most subtypes of HIV-1 and HIV-2 with an excellent specificity and sensitivity. Although PCR and other assays are available for the detection of viral nucleic acid, such assays are not as suitable for large scale use as are assays for antibodies.

It is commonly assumed that the "immunodominant" region of the transmembrane glycoprotein (gp41) is immunologically cross-reactive between all HIV-1 subtypes, and that a detectable antibody response to that region is induced in essentially all infected individuals. This appears to be the case with the well known subtypes A to G of HIV-1 (the classical group now designated group M), and there is no evidence at present that any of those subtypes behave differently from each other in any way when tested on current blood screening kits.

Nevertheless, it is widely believed, and much evidence supports the view, that the epitopes of HIV which are important for diagnostic purposes are different from those which play a role in protective (virus neutralising) responses. Classification of HIV is not based on the epitopes important for diagnosis. It is hence thought unlikely that the characteristics leading to the current classification of HIV-1 will be directly reflected in the behaviour of the viruses in diagnostic assays.

This belief is reflected in the recent finding that a strain of HIV-1, previously described as subtype O and now classified as group O, has proven difficult to detect with many current anti-HIV assays. It appears that group O samples behave differently on immunoassays from different manufacturers, and it has been found consistently that such samples are very

poorly detected in those assays based solely on HIV peptides.

As indicated above, HIV-1 group O was previously classified as a subtype of the classical HIV-1 (now called group M), but has been reclassified as a separate group (P. Charneau et al. Virology 205 (1994) 247-253). It is becoming increasingly clear that HIV-1 group O is not a diagnostic entity. The divergence within the group is becoming more apparent, as shown by the entries in the Los Alamos HIV database. The current classification tree for group O, which is based on the V3 loop, is already as branched as that for the classical HIV-1 (group M). It is thus becoming accepted that HIV-1 group O is a very diverse group.

The present invention provides an antigen that comprises or consists of a peptide SEQ. ID. NO. 1, designated MDL061, having the following sequence:

DRQISNISSTIYEEIQKAQVQQEQNEKKLLELD

or that has the antigenic properties of the peptide of SEQ. ID. NO. 1.

The amino acid sequence of the peptide of SEQ. ID. NO. 1 was derived from the amino acid sequence of the envelope of an HIV-1 virus, designated as ANT-70 (M. v.d. Haefevelde et al J. Virology 68 (1994) 1586-1596). ANT-70 is classified as HIV-1 group O on the basis of its V3 loop and LTR regions. ANT-70 has a region easily recognisable as homologous to the immunodominant region of other HIV-1 subtypes. Other portions of the envelope sequence vary in greater or lesser degree from the sequences of the common HIV-1 subtypes. The peptide of SEQ. ID. NO. 1 (MDL061) corresponds to amino acids 632 to 664 of the published ANT-70 envelope sequence.

A further peptide, SEQ.ID. NO. 2, designated MDL056, having the following sequence:

LETLLQNQQLLSLWGCKGKLVC

was produced. That peptide corresponds to residues 585 to 606 of the published ANT-70 envelope sequence, that is to say, to the presumptive immunodominant region of gp41 of ANT-70.

A similar peptide derived from the presumptive immunodominant region of the gp41 of an HIV-1 group O strain designated MVP-5180/91 (ECACC deposit no. V 920 92 318) is described in EP-0 591 914-A, and its use in the diagnosis of HIV is proposed.

It would be predicted, on the basis of the performance of corresponding peptides from the immunodominant region of gp41 of subtypes of classical HIV-1 (group M), that the peptide of SEQ. ID. NO. 2 would react with antibodies present in samples from substantially all individuals infected with HIV-1 group O.

However, when the peptide of SEQ. ID. NO. 2 was tested against a panel of samples of serum known to be HIV-1 positive and classified as HIV-1 group O positive by other methods, for example, by a negative result on testing with a competitive HIV-1 assay and a negative result on testing with an HIV-2 assay, it was found that the peptide did not react with all samples tested. It can be predicted, on the basis of the performance of the presumptive immunodominant peptide of SEQ. ID. NO. 2 that analogous peptides derived from the classical (presumptive) immunodominant region of gp41 of other strains of HIV-1 group O, for example, as described in EP-0 591 914-A, will also not detect all HIV-1 group O positive samples.

These results show that the use of an antigen derived from the classical (presumptive) immunodominant region of gp41 of HIV-1 group O will not enable effective and reliable detection of HIV-1 group O antibodies and hence infection with HIV-1 group O. That is surprising and in direct contrast to experience

with the subtypes of classical HIV-1 (group M). It is also in contrast to experience with subtypes (A to D) of HIV-2, all of which cross-react with the immunodominant region of the transmembrane protein gp36 of HIV-2 subtype A.

We found that when the peptides of SEQ. ID. NO. 1 and SEQ. ID. NO. 2 were tested against the panel of serum samples described above, neither peptide reacted with all samples; each reacted with a different selection of the samples. However, none of the presumed HIV-1 group O positive samples tested was unreactive to both peptides. The use of an antigen of the present invention in association with the use of a peptide derived from the classical (presumptive) immunodominant region of gp41 of HIV-1 group O, for example, a peptide of SEQ. ID. NO. 2 or an antigen as described in EP-0 591 914-A, will therefore ensure that all HIV-1 group O positive samples are detected.

The antigens used currently in HIV-1 assays enable the efficient detection of HIV-1 subtypes other than those now classified as group O (previously classified as subtype O). The results described above demonstrate that the incorporation of an antigen of the present invention together with an antigen derived from the classical (presumptive) immunodominant region of gp41 of HIV-1 group O, for example, a peptide of SEQ. ID. NO. 2 or an antigen as described in EP-0 591 914-A, in an assay for HIV-1 antibodies will result in an assay that will detect samples containing any and all currently known HIV-1 subtypes and groups, including group O. The use, in addition, of HIV-2 antigen(s) affords an effective assay for HIV-1 and HIV-2 antibodies.

Accordingly, the present invention provides an assay for determining HIV-1 antibodies in a test sample, which assay comprises contacting antibodies in the test sample with an HIV-1 antigen of the present invention, that is to say, comprising or consisting of a peptide SEQ. ID. NO. 1 r having the

antigenic properties of the peptide of SEQ. ID. NO. 1, and determining any antibody-antigen complex formed.

Preferably, an antigen comprising or consisting of or derived from an amino acid sequence of the presumptive immunodominant region of the transmembrane glycoprotein gp41 of a strain of HIV-1 group O is also used in the assay of the invention. The use of the two HIV-1 group O antigens ensures that all HIV-1 group O positive samples will be detected.

An antigen comprising or consisting of or derived from an amino acid sequence of the presumptive immunodominant region of the transmembrane glycoprotein gp41 of a strain of HIV-1 group O is, for example, a peptide of SEQ. ID. NO. 2 or that has the antigenic properties of the peptide of SEQ. ID. NO. 2. A further example of a suitable antigen is a peptide derived from the presumptive immunodominant region of the gp41 of an HIV-1 group O strain designated MVP-5180/91 (ECACC deposit no. V 920 92 318), as described in EP-0 591 914-A. A peptide of SEQ. ID. NO. 2 may be modified as described below for a peptide of SEQ. ID. NO. 1.

In order to enable reliable detection of other strains of HIV-1, one or more HIV-1 antigens from HIV-1 other than group O should also be used in the assay of the invention. Analogously, to enable detection of HIV-2, one or more HIV-2 antigens may be incorporated in the assay of the present invention.

All currently known subtypes (A to G) of the classical HIV-1 (group M) cross-react with antigens derived from the immunodominant region of group M HIV-1 gp41. Accordingly, an antigen comprising or consisting of or derived from an amino acid sequence of the immunodominant region of the transmembrane glycoprotein gp41 of a strain of HIV-1 group M may be incorporated in the assay of the invention. Other and/or further HIV-1 antigens may be incorporated as required or desired. For example, if and when new strains of HIV-1 arise

that do not cross-react with the immunodominant antigen of classical HIV-1 (group M), the appropriate antigen(s) may be incorporated in the assay.

As indicated above, the assay of the present invention may also comprises one or more HIV-2 antigens to enable detection of HIV-2 antibodies. The currently known subtypes (A to D) of HIV-2 all cross-react with the immunodominant region of the transmembrane protein gp36 of HIV-2 subtype A. An antigen comprising or consisting of or derived from an amino acid sequence of the immunodominant region of the transmembrane glycoprotein gp36 of HIV-2, for example, of subtype A may be used in the assay of the present invention. Other and/or further HIV-2 antigens may be incorporated as required or desired.

Antibodies in the test sample may be contacted with the antigen(s) used in the assay directly, by contacting the test sample with the antigens immobilised on a solid phase, or by contacting the test sample with the antigen(s) in a homogeneous phase. Using a different format, the test sample may be contacted with a solid phase on which is immobilised means capable of binding antibodies non-selectively. The captured antibodies are then contacted with the antigen(s). In each case, any specific antibody-antigen complex formed is detected. Further details of methods of carrying out the assay of the invention are given below.

A peptide of SEQ. ID. NO. 1 (MDL061) may be modified in any of the ways known in the art providing it retains its function as an HIV-1 antigen, that is to say, the immunological, in particular the antigenic, characteristics of the peptide itself are retained. All such modified versions of the peptide are themselves antigens of the present invention.

A simple test for determining whether an antigen that is modified version of a peptide of SEQ. ID. NO. 1 retains its

antigenic function (the immunological characteristics of the peptide itself) is to test that antigen for immunological reactivity with antibodies that bind to the peptide of SEQ. ID. NO. 1 itself. It is convenient to use a sample or, preferably, a panel of samples that have been shown to react with the peptide of SEQ. ID. NO. 1 itself.

Any one or more of the following modifications may be carried out:

Amino acids within the peptide may be substituted by other amino acids, especially by conservative substitutions, provided the function of the peptide is retained. The peptide may be shortened, at either or both ends or by removal of an internal sequence.

Additional residues for example, cysteine or serine may be incorporated, for example, at the N-terminus of an antigen of the invention, to assist linking the antigen to other moieties.

The peptide may be elongated, and may form part of a larger (poly)peptide. The larger (poly)peptide may be a recombinant (poly)peptide or a naturally-occurring (poly)peptide.

It may be advantageous to form a fusion polypeptide comprising an antigen of the invention and one or more other antigens, especially HIV-1 and/or HIV-2 antigens. For example, a fusion polypeptide may comprise an antigen of the invention and one or more antigens selected from
(i) the peptide SEQ. ID. NO. 2 (MDL056) and any other antigen derived from or comprising the amino acid sequence of the classical (presumptive) immunodominant region of a strain of HIV-1 group O, (ii) one or more other HIV-1 antigens (peptides or polypeptides) and
(iii) one or more HIV-2 antigens (peptides or polypeptides).

A fusion protein of the present invention may comprise a

peptide of the invention, any other HIV antigens present, and a non-HIV component. The non-HIV component may be a carrier enzyme, for example, ß-galactosidase or superoxide dismutase. A fusion protein may comprise an antigen derived from another pathogen, for example, hepatitis B surface or core antigen. A fusion protein may comprise a component useful in antibody production or to increase immunogenicity, for example, phytohaemagglutinin.

A peptide antigen of the invention may be produced by chemical synthesis, for example, by the classical Merryfield method, or by any other method suitable for synthesising peptides of the appropriate length. Automated equipment is available for chemical synthesis of peptides.

If desired, a peptide may be produced by recombinant methods in either a eukaryotic or a prokaryotic host. Relative advantages and disadvantages of eukaryotic and prokaryotic systems are known and the choice of system will depend on the balance of various factors in each case. Recombinant methods may be particularly useful if the peptide is elongated or is part of a polypeptide, for example, a fusion protein.

Methods for producing recombinant proteins are well known and are described, for example, in Sambrook J., Fritsch E.F., Maniatis T. Molecular cloning: A laboratory manual, Cold Spring Harbor 1989.

An antigen of the present invention, for example a peptide or polypeptide, may be obtained from a natural source, for example, from a lysate of a strain of HIV-1 group O.

There are well-known advantages and disadvantages in the use of synthetically-produced peptide antigens, recombinant antigens and antigens obtained from natural sources. Advantages of synthetic peptides over natural antigens include removal of the risk of infection and advantages of synthetic peptides over

recombinant antigens include elimination of potentially cross-reacting antigens, from example, host cell antigens, from the recombinant production system.

As indicated above, the terms "antigen of the invention" and "(poly)peptide of the invention" and "MDL061" include not only the peptide of SEQ. ID. NO. 1 set out above but also all modified versions of that peptide, for example, as described above, provided that the immunological, in particular the antigenic characteristics of the modified version of the peptide is essentially the same as that of the peptide itself.

For example, as shown below, a majority of classical HIV-1 samples (70 of 96 tested) react with peptide MDL056, SEQ. ID. NO. 2 whereas none of those samples reacts with the peptide of SEQ. ID. NO. 1 (MDL061). Modified versions of the peptide of SEQ. ID. NO. 1 (MDL061) should, for example, reflect the immunological characteristics of the peptides MDL056 and MDL061 demonstrated with that panel of samples, or with any other panel of samples.

As indicated above, an antigen of the present invention may be used in any form of immunoassay assay for HIV antibodies, either in a homogenous phase assay or in a capture (heterogenous phase) assay. In a homogeneous phase assay, any antibody-antigen complex formed is detected in solution without separation of the phases. Such assays are generally competitive assays, and an antigen of the invention is used in both labelled and unlabelled form.

In a capture assay, an antibody-antigen complex between an antigen of the invention and any corresponding antibody present in a test sample may be formed in several different ways: an antigen of the invention may be immobilized on a solid surface to capture selectively the corresponding antibodies; an antigen of the invention may be used to label antibodies already captured; or an antigen of the invention may be used both to

capture and to label antibodies. Means for determining an antibody-antigen complex in a capture assay may be incorporated in the antigen itself, for example, a directly or indirectly labelled antigen may be provided, or the means may be part of a separate directly or indirectly labelled entity. Such means are well known in the art, and are described by way of example in more detail below.

Numerous books and review articles describe the theory and practice of immunoassays. Advice is given on the design of immunoassays, for example, on the choice of homogeneous or capture format, on the characteristics and choice of solid substrate in the case of capture assays, on the nature and choice of label (signal generating system), and on various other practical matters such as the composition of diluents and washing solutions. An example of a standard textbook is "ELISA and Other Solid Phase Immunoassays, Theoretical and Practical Aspects", Editors D.M. Kemeny & S.J. Challacombe, pub. John Wiley, 1988.

The test sample under investigation is generally blood (plasma or serum), saliva or urine, but other liquid samples, for example, cerebro-spinal fluid, joint fluid, sweat or tears may be used, as may liquid samples derived from solid tissue, for example, tissue exudate.

In general, in homogeneous assays the antibody and the antigen are both labelled so that, when the antibody and antigen interact in solution, the two labels also interact, for example, to allow non-radiative transfer of energy captured by one label to the other label, with appropriate detection of the excited second label or quenched first label, for example, by fluorimetry, magnetic resonance or enzyme measurement. Addition of antibody in a sample under investigation results in modification of the interaction of the labelled pair and so to a different level of signal. In many cases, the signal is preferably a colour change.

In a capture assay, the solid phase on which the capture occurs should be capable of being washed in some way and is, for example, of a plastics or polymeric material, for example, of nitrocellulose, polyvinyl chloride, polystyrene, polyamide, polyvinylidine fluoride or other synthetic polymers. The solid phase may be formed into microtitre wells, beads, dipsticks, aspiration tips, electrodes and optical devices. The solid phase may be particles, for example, so-called "latex" particles, stabilised blood or red blood cells, bacterial or fungal cells, spores, gold or other metallic sols, and proteinaceous colloids. The size of particles is generally from about 0.02 to 5 microns, and beads generally larger, for example, from about 2 mm to 10 mm. Alternatively, the solid phase may be a slide or "tile" having coated hollows. Slides, cards and tiles may be used as immunoassay vessels for agglutination assays.

Microtitre plates and beads of polymeric material are particularly convenient, especially for large-scale use, since automated systems are available for handling such test formats.

Other solid phases that may be used include membranes, sheets and strips, for example, of a porous, fibrous or bibulous material, for example, of nylon, polyvinyl chloride or another synthetic polymer, of a natural polymer, for example, cellulose, of a derivatized natural polymer, for example, cellulose acetate or nitrocellulose, or of glass fibres. Paper, for example, diazotized paper may be used. Films and coatings, for example, of fibrous or bibulous material, for example, as described above, may be used as the solid phase.

It is to be understood that the above examples of solid phases are given by way of illustration only, and the invention is not limited to the use of such solid phases. The invention may be practised on any solid phase suitable for use in immunoassays.

A solid phase, for example, a membrane, sheet, strip, film or

coating, may be incorporated in a device for the determination of multiple or, more generally, single samples.

The term "assay device" is used herein to denote means for carrying out an immunoassay comprising a solid phase, generally a laminar solid phase, for example, a membrane, sheet, strip, coating, film or other laminar means, on which are immobilized antibodies to one or more classes of immunoglobulin. The immobilized antibodies are preferably present in a defined zone, called herein the "antigen capture zone".

An assay device may incorporate the solid phase within a rigid support or a housing, which may also comprise some or all of the reagents required for carrying out an assay. Sample is generally applied to an assay device at a predetermined sample application zone, for example, by pouring or dripping the sample on the zone, or by dipping the relevant part of the device into the sample. If the sample application zone is at a different site from the antibody capture zone, the arrangement of the device is generally such that antibodies in the sample migrate to the antibody capture zone. The required reagents are then applied in the appropriate order at designated application zones, which may or may not be the same as the sample application zone. Again, if the or any reagent application zone is at a different site from the antibody capture zone, the arrangement of a device is generally such that the reagent(s) migrate to the antibody capture zone, where any antigen-antibody complex formed is detected. All or some of the reagents required for an immunoassay may be incorporated within a device, in liquid or dry form. If so, a device is generally arranged such that interactions between different parts of the device, which interactions may occur automatically during the operation of the device or may be brought about by the user of the device, bring the various reagents into contact with one another in the correct sequence for the immunoassay to be carried out.

A wide variety of assay devices are described in the literature of immunoassays. Examples of membrane devices are described in U.S. Patents Nos. 4,623,461 and 4,693,984. Depending on their design and their speed of action, some assay devices are called "dipsticks" and some are called "rapid assay" devices. A "rapid assay" device generally provides a result within ten minutes of the application of sample. (A typical microtitre plate or bead assay requires incubation steps, and generally takes at least an hour to provide a result.) Accordingly, although assay devices are generally more expensive than microtitre or bead format assays, they have particular uses in clinical testing, for example, when a result is required rapidly, for example, in the case of emergency surgery.

Assay devices have the particular advantage that they can be used without the need for sophisticated laboratory facilities or even without the need for any laboratory facilities. They may therefore be used for "on the spot" testing, for example, in an emergency room, in a doctor's surgery, in a pharmacy or, in certain cases, for home testing. They are particularly useful in territories where laboratory facilities are few and far between.

A further type of immunoassay is a flocculation assay, which involves an immunological interaction between two components resulting in flocculation that may be determined by eye and/or may be determined photometrically. Microparticles, for example, microparticulate carbon, may be used to enhance the visual difference between a positive and negative reaction.

An antigen of the invention may be used to capture antibodies from the test sample. For the reasons given above, it is advantageous to use both an antigen of the invention and an antigen derived from or comprising an amino acid sequence from the presumptive immunodominant region of gp41 of HIV-1 group O for capture.

An antigen of the invention, preferably in admixture with a presumptive immunodominant HIV-1 group O antigen, may be coated on the solid phase according to known methods. The captured antibodies may then be detected either using labelled antigen(s) according to the invention or by using any entity that will bind to the captured antibody, for example, protein A or protein G; an antispecies immunoglobulin or an anti-immunoglobulin sub-type, for example, an anti-IgG antibody; or an antibody to the antigen(s) used in competitive or blocking manner; or any molecule containing an epitope contained in the antigen(s). Suitable entities and suitable labels are well known, and labelling systems are described in more detail below.

As an alternative to coating the solid phase with antigen(s) according to the invention to capture selectively the antibodies of interest, the solid phase may be coated with any substance(s) capable of capturing antibodies non-selectively. A representative proportion of all antibodies is captured, and then labelled antigens(s) according to the invention are used to detect those captured antibodies that are directed against those particular peptide(s).

Suitable capture entities include protein A, protein G, anti-Fc-antibodies, anti-IgG and anti-IgM antibodies. It is particularly advantageous to use a mixture of anti-IgG and anti-IgM since then both early and late antibodies are captured. That is particularly important in the case of HIV, especially for screening donated blood.

For determination of captured antibody, each or any antigen used according to the invention, for example, an antigen of the invention, another HIV-1 group O antigen or any other HIV-1 or HIV-2 antigen, may itself be labelled with means capable directly or indirectly of providing a detectable signal to enable any antibody-antigen complex to be detected. The labelled antigen is called an "antigen conjugate".

The detectable signal may be optical, radio-active or physico-chemical, and may be provided directly by labelling the antigen for example, with a dye, coloured particle, radiolabel, electroactive species, magnetically resonant species or fluorophore; or indirectly by labelling the antigen with an enzyme itself capable of giving rise to a measurable change of any sort. Alternatively, the signal may result from agglutination, a diffraction effect or a birefringement effect involving the antigen conjugate.

In a further embodiment, either when the antigen of the present invention is a peptide or is part of a larger polypeptide, for example, as a fusion protein, the means capable indirectly of providing a detectable signal comprises an antibody that binds to the larger antigen. That antibody (Ab1) may be provided with direct or indirect label means as described above for the antigen (single antibody detection system), or it may be labelled by means of yet another antibody (Ab2), which binds to Ab1 and is itself labelled directly or indirectly as described above for the antigen (dual antibody detection system). A single or dual antibody system for detection of captured anti-body-antigen complex is particularly useful if it is difficult to produce a suitable antigen conjugate. (Some conjugates, for example, tend to be insufficiently stable for commercial use, and some antigens lose antigenicity on conjugation.)

The single antibody detection system has the advantage of involving fewer washing and incubation steps, but the disadvantage that it is necessary to produce an antibody conjugate (labelled antibody Ab1) for each antigen to be detected. The dual antibody detection system has the advantage that, with appropriate choice of Ab1 and Ab2, it is possible to use one antibody conjugate (labelled antibody Ab2) for the detection of all captured antibodies as follows: when each antibody (Ab1) is raised in the same animal species, the second antibody (Ab2) can be an anti-species antibody. For example, if each antibody Ab1 is a sheep antibody, then a labelled anti-

sheep antibody can be used as Ab2. The advantage to the user of requiring only one antibody conjugate for detection of all analytes generally outweighs the disadvantage of the need for an extra incubation and extra washing steps, particularly when an automated system is used.

If the entity to be used for determination of captured antibody is other than an antigen of the invention, that entity may be labelled analogously to the labelling of the antigen, and may be labelled either directly or indirectly.

A labelling system that is particularly preferred for a antigen or other conjugate is an enzyme labelling system, particularly one in which the molecule is conjugated to an enzyme that catalyses a detectable colour change in the presence of a suitable substrate. This format, the enzyme-linked immunoassay or ELISA, is widely used commercially, and automated instruments are available for carrying out such assays using, for example, microtitre plates, the proprietary "bead" or "IMX" (Trade Mark) systems, or using hollow rods or pipette tips, and computer software is available to process the results obtained.

The enzyme systems used are well known per se, see for example, "ELISA and Other Solid Phase Immunoassays, Theoretical and Practical Aspects" Eds. Kemeny D.M. & Challacombe S.J.,

Examples of typical enzyme systems are those using alkaline phosphatase, ß-galactosidase, urease or peroxidase, for example, horse-radish peroxidase.

Labelling systems that are particularly useful in assay devices are those that give rise to a colour change detectable by eye, for example, involving the use of coloured particles.

In summary, antigens of the present invention may be used in assays for the determination of antibodies to HIV-1 in many different formats, including ELISA, competitive immunoassays,

membrane bound assays, including those incorporated in assay devices, and immunoprecipitation.

An assay of the invention may be a qualitative assay, for the detection of samples containing HIV-1 antibodies, or may be semi-quantitative or quantitative.

As indicated above, when the assay is to determine HIV-1 and, if desired, also HIV-2, then the appropriate antigens are incorporated in the assay. If the assay is a capture assay, further HIV antigens in addition to the antigens of the invention may be immobilized on the solid phase. The captured antibodies may be detected either by using labelled HIV antigens or by using another labelled component, for example, a labelled anti-species antibody, as described above. When multiple specifically captured antibodies are to be detected, as when specific antibodies are captured by immobilised antigens, it may be preferable to use a labelled component that will interact with all captured antibodies, for example, labelled anti-human antibodies. If the antibodies are captured non-specifically, for example, using immobilized anti-IgG, preferably in combination with immobilized anti-IgM, then the appropriate antigens, labelled directly or indirectly as described above, are preferably used for determination. In the case of non-specific antibody capture, the choice of labelled antigen will determine whether the test is a general HIV test (HIV-1 and HIV-2 antigens to be used) or a test for HIV-1 only (HIV-1 antigens only to be used).

The HIV-1 and HIV-2 antigens to be used in addition to an antigen of the invention may be any antigens described in the literature of the art (including the patent literature) or in actual use in the art. The antigens may be peptides or polypeptides, and may be produced by chemical synthesis or by recombinant DNA technology. The antigens include fusion proteins. An HIV-1 antigen is especially one comprising an amino acid sequence derived from the immunodominant trans-

membrane protein. For HIV-2, the antigen is preferably from the analogous region. Examples of suitable antigens are given in EP-A-0 307 149 and EP-A-0 347 148.

Components for carrying out an assay of the present invention are generally presented in the form of a kit

The present invention provides a kit for the determination of antibodies to HIV-1 in a test sample, comprising
(i) a solid phase on which is immobilized an antigen of the present invention, and
(ii) means for the detection of any captured antibody.

Preferably the solid phase also has immobilised an antigen derived from, comprising or consisting of an amino acid sequence from the presumptive immunodominant region of gp41 of HIV-1 group O. The solid phase may also have immobilized at least one other HIV-1 antigen, especially an HIV-1 antigen derived from other than HIV-1 group O, especially an antigen from the presumptive immunodominant region of such HIV-1. The solid phase may further comprise an immobilised HIV-2 antigen for the detection of HIV-2 antibodies.

The detection means may be labelled antigen(s) of the invention (and other labelled HIV antigens) or other labelled entities as described above, for example, labelled anti-human antibodies.

The solid phase on which is immobilised an antigen of the invention and optionally further antigen(s) as described above is itself part of the present invention.

The present invention also provides a kit for the determination of antibodies to HIV-1 in a test sample, comprising
(i) a solid phase on which is immobilized means for non-selectively capturing antibodies, and
(ii) an antigen of the present invention, said antigen being labelled, directly or indirectly, with a detectable label.

Preferably there is also provided a labelled antigen derived from, comprising or consisting of an amino acid sequence from the presumptive immunodominant region of gp41 of HIV-1 group O. There may also be provided at least one other labelled HIV-1 antigen, especially an HIV-1 antigen derived from other than HIV-1 group O, especially an antigen from the presumptive immunodominant region of such HIV-1. There may additionally be provided a labelled HIV-2 antigen for the detection of HIV-2 antibodies.

The present invention also provides isolated antibodies to an antigen of the invention, for example, polyclonal and especially monoclonal antibodies. Methods for producing polyclonal and monoclonal antibodies are well known.

The present invention also provides a nucleic acid encoding an antigen of the invention.

As indicated above, results we have obtained using an antigen of the invention (peptide MDL061, SEQ. ID. NO. 1) and an antigen from the presumptive immunodominant region of gp41 of HIV-1 group O (peptide MDL 056, SEQ. ID. NO. 2) in the investigation of samples classified as HIV-1 group O indicate that the designation "HIV-1 group O", while possibly meaningful from the viewpoint of molecular biology and for vaccine development is not generally useful for diagnostic purposes: some group O samples cross-react completely on classic commercial HIV kits; some samples do not. Some group O samples react with the presumptive immunodominant region of the group O virus (peptide MDL056, SEQ. ID. NO. 1); some do not. 70 of 96 classical HIV-1 samples react with the immunodominant sequence of group O (peptide MDL056). Some group O samples react with the MDL061 peptide; some do not, but none of 96 classical HIV-1 samples react with MDL061.

In view of these results showing differing cross-reactions and lack of cross-reactions, group O does not appear to be a

diagnostic entity. Furthermore, classification according to the V3 loop indicates considerable diversity within the group.

Nevertheless, if it should be desired to determine if samples that are HIV-1 negative according to conventional assays are HIV-1 group O samples, for example, for epidemiological studies, there may be used an HIV antibody assay that comprises, as antigen, an antigen of the present invention. Of course, antigens from HIV-1 other than group O should not be present. An antigen derived from the presumptive immunodominant region of gp41 of HIV-1 group O may be incorporated in the assay, if desired. Such HIV group O assays are part of the present invention. The assays may be homogeneous phase or heterogenous phase (capture) assays and may be carried out as described above in relation to the general HIV antibody assays of the present invention.

The following Examples illustrate the invention:

EXAMPLE 1

Production of peptides

The following peptides were synthesised as the free carboxy-terminal derivatives by the Fmoc method on a p-hydroxymethylphenoxymethyl (HMP) resin by stepwise solid phase synthesis according to the general procedure set out in Applied Biosystem User Bulletin No. 33, November 1990, using automated laboratory techniques:

CDRQISNISSTIYEEIQKAQVQQEQNEKKLLELD Peptide I

SLETLLQNQQLLSLWGCKGKLVC Peptide II.

Peptide I is MDL061 (SEQ. ID. NO. 1) with the addition of an N-terminal cysteine residue to assist subsequent reactions to form a conjugate (to add a label to the peptide). An N-terminal serine reside was added to MDL056 (SEQ. ID. NO. 2) for the same reason, giving peptide II.

EXAMPLE 2

Immunoassay on HIV-1 group O samples

The wells of a microtitre plate were coated passively with peptide MDL061 (SEQ. ID. NO. 1) or with peptide MDL056 (SEQ. ID. NO. 2).

The test samples were eleven sera obtained from patients classified as being infected with HIV-1 group O. Of the eleven samples, two sera (2 and 11) are different bleeds from the same individual. None of the sera gave a positive result on a competitive HIV-1 EIA (Wellcozyme anti-HIV-1), which is one criterion used currently to indicate lack of infection by a classical HIV type or group (but not ruling out the possibility of infection by a non-classical HIV-1 subtype or by HIV-2). The sera had been tested for antibodies to HIV-2 and were all negative.

The test samples were incubated in the microwells, which were then washed and incubated with anti-human IgG/peroxidase conjugate and finally with substrate for the peroxidase. Control assays using unrelated peptides immobilized on other microtitre plates showed that any binding that occurred was specific. The results of the assay are given in Table 1 below:

TABLE 1

Sample	MDL056	MDL061
1	0.643	over
2	over	0.234
3	0.182	over
4	0.08	over
5	over	over
6	1.7	over
7	0.25	0.547
8	0.338	1.118
9	1.454	0.196
10	1.869	0.169
11	over	0.317

The values tabulated are optical densities. "Over" denotes that the optical density was above the range of the reader. The "cut-off" is 0.3 O.D. above the negatives.

It can been seen that neither of the peptides reacted with all the sera thus neither is immunodominant. No sample is unreactive to both peptides. A combination of the two peptides may therefore be used to detect all HIV-1 group O samples.

EXAMPLE 3

Immunoassays on HIV-1 positive samples

Immunoassays were carried out as described in Example 2 above except that the sera used were 96 samples shown by the competitive HIV-1 assay described above to be HIV-1 positive but not group O by the above definition. In addition to assays using immobilized peptides MDL061 (SEQ. ID. NO. 1) and MDL056 (SEQ. ID. NO. 2), an assay was carried out by the same method using immobilized peptide AC056, which corresponds to amino acids 583 to 604 (from the immunodominant gp41 region) of the HIV-1 isolate CBL-1.

	MDL056	MDL061	AC056
Number reactive	70	0	95
Number unreactive	26	96	1

"Reactive" means having an O.D. value greater than 0.30 above the mean value of sera from uninfected individuals on a plate coated with the same peptide.

It will be seen that samples which react strongly positive on competitive HIV-1 EIA (i e. are not group O) and which react with the immunodominant region of a classical strain of HIV-1 (peptide AC056) do not react on peptide MDL061. Many of those sera react with MDL056, which thus can be considered to mimic the classical (presumptive) immunodominant region of both group O and classical HIV-1. This data shows that the designation "HIV-1 group O" while possibly being meaningful from a

molecular biological and vaccine development standpoint is not generally useful from a diagnostic standpoint. Some group O samples cross-react completely on classical commercial HIV kits. Some group O samples do not. Some group O samples react with the expected immunodominant sequence of the group O virus, some do not. Some group O samples react with the MDL061 sequence, some do not; but none of 96 classical HIV-1 samples react with MDL061. 70 of 96 classical HIV-1 samples react with the immunodominant sequence of group O.

In view of these differing cross-reactions and lack of cross-reactions group O does not appear to be a diagnostic entity.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: INTERNATIONAL MUREX TECHNOLOGIES CORPORATION
(B) STREET: 3075 Northwoods Circle
(C) CITY: Norcross
(D) STATE: Georgia
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 30071

(ii) TITLE OF INVENTION: ASSAY FOR HIV-1 GROUP O

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9410044.3
(B) FILING DATE: 19-MAY-1994

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1
(C) INDIVIDUAL ISOLATE: ANT-70

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asp Arg Gln Ile Ser Asn Ile Ser Ser Thr Ile Tyr Glu Glu Ile Gln
1 5 10 15

Lys Ala Gln Val Gln Gln Glu Gln Asn Glu Lys Lys Leu Leu Glu Leu
20 25 30

Asp

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1
(C) INDIVIDUAL ISOLATE: ANT-70

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Ser Leu Trp Gly Cys
1 5 10 15

Lys Gly Lys Leu Val Cys
20

CLAIMS:

1. An antigen that comprises or consists of a peptide SEQ. ID. NO. 1, having the following sequence:

DRQISNISSTIYEEIQKAQVQQEQNEKKLLELD

or that has the antigenic properties of the peptide of SEQ. ID. NO. 1.

2. An assay for determining HIV-1 antibodies in a test sample, which assay comprises contacting antibodies in the test sample with an antigen as claimed in claim 1, and determining any antibody-antigen complex formed.

3. An assay as claimed in claim 2, wherein antibodies in the test sample are also contacted with at least one other HIV antigen, and any antibody-antigen complex formed is determined.

4. An assay as claimed in claim 3, wherein the or one of the other HIV antigens is an antigen comprising or consisting of or derived from an amino acid sequence of the presumptive immunodominant region of the transmembrane glycoprotein gp41 of a strain of HIV-1 group O.

5. An assay as claimed in claim 3 or claim 4, wherein one of the other HIV antigens is an HIV-1 antigen of HIV-1 other than group O.

6. An assay as claimed in claim 5, wherein the HIV-1 antigen of HIV-1 other than group O is an antigen comprising or consisting of or derived from an amino acid sequence of the immunodominant region of the transmembrane glycoprotein gp41 of a strain of HIV-1 other than group O.

7. An assay as claimed in any one of claims 3 to 6, wherein one of the other HIV antigens is an HIV-2 antigen.

8. An assay as claimed in claim 7, wherein the HIV-2 antigen is an antigen comprising or consisting of or derived from an amino acid sequence of the immunodominant region of the transmembrane glycoprotein gp36 of a strain of HIV-2.

9. A kit for the determination of antibodies to HIV-1 in a test sample, comprising
(i) a solid phase on which is immobilized an antigen of the present invention, and
(ii) means for the detection of any captured antibody.

10. A kit as claimed in claim 9, wherein the solid phase also comprises one or more further antigens as defined in any one of claims 4 to 8.

11. A kit for the determination of antibodies to HIV-1 in a test sample, comprising
(i) a solid phase on which is immobilized means for non-selectively capturing antibodies, and
(ii) an antigen of the present invention, said antigen being labelled, directly or indirectly, with a detectable label.

12. A kit as claimed in claim 11, which also comprises one or more further labelled antigens as defined in any one of claims 4 to 8.

13. A solid phase on which is immobilised an antigen as claimed in claim 1 and optionally one or more further antigens as defined in any one of claims 4 to 8.

14. An antibody to an antigen as claimed in claim 1, being a mon clonal antibody or substantially isolated polyclonal antibodies.

15. A nucleic acid that encodes an antigen as claimed in claim 1.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB 95/01146

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/48 C07K14/16 C07K16/10 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 591 914 (BEHRINGWERKE AG) 13 April 1994 cited in the application SEQ ID No. 46 see claims 1,4,7,13-16,18-23; examples 7,8; table 3 ---	1,2,9,15
X,Y	JOURNAL OF VIROLOGY, vol. 68, no. 3, March 1994 pages 1586-1596, M. VANDEN HAESEVELDE ET AL. 'Genomic Cloning and Complete Sequence Analysis of a Highly Divergent African Human Immunodeficiency Virus Isolate' cited in the application see page 1593, right column, paragraph 4; figure 2 see page 1594, left column, paragraph 4 --- -/--	1,2,9-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 September 1995	17. 10. 95

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

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PCT/GB 95/01146

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 335 134 (ABBOTT LAB) 4 October 1989 see page 13, line 5 - page 14, line 19; claims	1,2,9-15
A	WO,A,93 18054 (INNOGENETICS NV) 16 September 1993 see page 8, paragraph 1 see page 8, paragraph 7 see page 24, paragraph 1 - page 26, last paragraph; claims; examples	1,3,9
A	EP,A,0 345 375 (INNOGENETICS NV) 13 December 1989 see page 23, line 29 - page 24, line 38; claims; examples	1-9

INTERNATIONAL SEARCH REPORT

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Interr ial Application No
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